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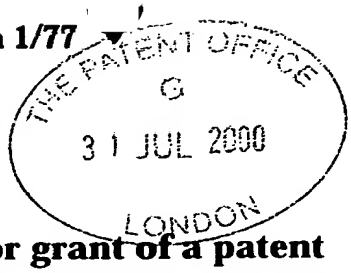

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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
   Mars UK Limited
   3D Dundee Road
   Slough
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   Patents ADP number *(if you know it)* 07475973001

   If the applicant is a corporate body, give the country/state of its incorporation: UK

4. Title of the invention

5. Name of your agent *(if you have one)*

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*
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   20 Red Lion Street
   London WC1R 4PJ

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
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Description 36

Claim(s) 6

Abstract 0

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Antioxidant Compositions and Methods for Companion Animals

Free radicals are inherent in the aerobic metabolism of living organisms and are generated by both physiological and pathological processes. They are sometimes generated intentionally to serve biological functions, such as microbicides in phagocyte cells, or may be accidents of chemistry following which they exhibit destructive behaviours. Whatever their mechanism of generation, if free radical production and removal is not controlled, then their effects on an organism can be damaging. To combat excessive and inappropriate damage, an elaborate system of antioxidant defences has evolved.

When there is an unbalance between the oxidants and the antioxidants in favour of the oxidants, a condition of oxidative stress exists that can lead to tissue damage.

Vaccinations represent approximately 25% of the total veterinary medicine market and with the recent introduction of 'Pet Passports' in the United Kingdom and the associated vaccination requirements, this percentage is destined to grow at least in the area of companion animals. Domestic cats in the United Kingdom are vaccinated annually against calicivirus, amongst other viruses and likewise dogs are immunised annually against a number of pathogens including parvovirus and distemper. Both cats and dogs may be further vaccinated against the rabies virus if a Pet Passport is required. Accompanying this growth is an increase in reports of adverse vaccine reactions and growing owner concern regarding the safety of vaccinations in their pets. Veterinary drug companies are now addressing these concerns in a number of ways; separating vaccines which were previously given in combination (ensuring the animal gets only the vaccine it needs), and investigating new methods of vaccine delivery (e.g. oral vaccinations through transgenic food crops, needle-free transcutaneous vaccination, novel adjuvants). An important consideration in the development of new vaccines is of course efficacy.

The present invention provides, amongst others, a means to overcome the problem of oxidative stress in the domestic cat and dog. The present invention also provides means for enhancing vaccine efficacy, in a particularly safe and easy way, through nutrition.

Accordingly, a first aspect of the invention provides a method for increasing the plasma vitamin E level in a domesticated cat or dog, the method comprising the step of administering to said cat or dog, an amount of vitamin E sufficient to increase the plasma vitamin E level. The increase may be to the maximum/saturation point measurable in the plasma of the animal. The increase may be in the range of 2 to 3 times the animal's own base line for plasma vitamin E levels (around the maximum physiological increase). The increase may be measured as an increase in the plasma vitamin E level of up to 25%, preferably 25% or above (preferably up to 50%, or 25 to 50%, or even 50 to 90%) of an individual animal when compared to the plasma vitamin E level when the animal is fed a control diet. The control diet, for example, is such that the total vitamin E consumption for the cat or dog is 10IU/400kcal.

Vitamin E is a collective term for several biologically similar compounds, including those called tocopherols and tocotrienols, which share the same biological activity. The most biologically active biological form of vitamin E (also the most active antioxidant) in animal tissue is alpha-tocopherol. Vitamin E cannot be synthesised *in vivo*. Vitamin E protects against the loss of cell membrane integrity, which adversely alters cellular and organelle function.

Units of vitamin E can be expressed as International Units (IU), where 1 IU of alpha-tocopherol equals 1mg of alpha-tocopherol. Other vitamin E compounds have their IU determined by their biopotency in comparison to alpha-tocopherol as described in McDowell, L.R (1989) Vitamin E: In vitamins in Animal Nutrition, Chapter 4, page 96, Academic Press, UK.

To date, levels of vitamin E above and beyond the minimum levels sufficient to prevent vitamin E deficiency symptoms present in the domestic dog or cat have not been of interest. This invention identifies that the levels of vitamin E in the dog or cat reflect the levels present in their diet and that these levels provide a typical baseline level (see examples) which is not exceeded when the animal is fed on prepared petfood. The present invention shows that the levels of vitamin E in the dog and cat can be increased by incorporating higher levels of vitamin E in the animal's diet (and that this can be achieved by the provision of specialised prepared petfood and/or a cat or dog supplement).

Aspects of the invention provide a means for reducing oxidative stress in the domestic cat and dog. Such a reduction in oxidative stress, in particular strengthens the immune response and provides a healthier animal. Markers of oxidative damage in a dog or cat include, amongst others: plasma carbonyls (end products of protein oxidation), plasma lipid hydroperoxides (markers of lipid oxidation), and anti-LDL antibodies which are produced as a response to LDL oxidation. A decline in any of these is indicative of reduced oxidative damage.

The vitamin E according to the first aspect of the invention may be in any form. It may be a tocopherol or a tocotrienol. It may be alpha-tocopherol, (d-α or dl-α) beta-tocopherol (d-β or dl-β), gamma-tocopherol (d-γ or dl-γ), delta-tocopherol, alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol or delta-tocotrienol. Preferably it is alpha-tocopherol.

The source of the vitamin E is not limiting. Preferred vitamin E sources include vitamin E acetate, (e.g tocopherol acetate), vitamin E acetate adsorbate or vitamin E acetate spray dried. Preferred sources are synthetic although natural sources may be used.

The form of administration of the vitamin E is not limiting. It may be in the form of a diet, foodstuff or a supplement. Hereinafter in this text, the term "foodstuff" covers all of foodstuff, diet and supplement. Any of these forms may be solid, semi-sold or liquid.

The supplement is particularly useful to supplement a diet or foodstuff which does not contain sufficiently high levels of one or more of the components according to the invention. The concentrations of the components in the supplement may be used to "top up" the levels in the animal's diet or foodstuff. This can be done by including a quantity of the supplement with the animal's diet or by additionally feeding the animal a quantity of the supplement. The supplement can be formed as a foodstuff with extremely high levels of one or more components of the invention which requires dilution before feeding to the animal. The supplement may be in any form, including solid (e.g. a powder), semi-solid (e.g. a food-like consistency/gel), a liquid or alternatively, it may be in the form of a tablet or capsule. The liquid can conveniently be mixed in with the food or fed directly to the animal, for example via a spoon or via a pipette-like device. The supplement may be high in one or more components of the invention or may be in the form of a combined pack of at least two parts, each part containing the required level of one or more component.

Preferably the vitamin E is incorporated into a commercial petfood product or a commercial dietary supplement. The petfood product may be a dry, semi-dry, a moist or a liquid (drink) product. Moist products include food which is sold in tins or foil containers and has a moisture content of 70 to 90%. Dry products include food which have a similar composition, but with 5 to 15% moisture and presented as biscuit-like kibbles. The diet, foodstuff or supplement is preferably packaged. In this way the consumer is able to identify, from the packaging, the ingredients in the food and identify that it is suitable for the dog or cat in question. The packaging may be metal (usually in the form of a tin or flexifoil), plastic, paper or card. The amount of

moisture in any product may influence the type of packaging which can be used or is required.

The foodstuff according to the present invention encompasses any product which a dog or cat may consume in its diet. Thus, the invention covers standard food products, as well as pet food snacks (for example snack bars, biscuits and sweet products). The foodstuff is preferably a cooked product. It may incorporate meat or animal derived material (such as beef, chicken, turkey, lamb, blood plasma, marrowbone etc, or two or more thereof). The foodstuff alternatively may be meat free (preferably including a meat substitute such as soya, maize gluten or a soya product) in order to provide a protein source. The product may contain additional protein sources such as soya protein concentrate, milk proteins, gluten etc. The product may also contain a starch source such as one or more grains (e.g. wheat, corn, rice, oats, barely etc) or may be starch free. A typical dry commercial dog and cat food contains about 30% crude protein, about 10-20% fat and the remainder being carbohydrate, including dietary fibre and ash. A typical wet, or moist product contains (on a dry matter basis) about 40% fat, 50% protein and the remainder being fibre and ash. The present invention is particularly relevant for a foodstuff as herein described which is sold as a diet, foodstuff or supplement for a cat or dog.

In the present text the terms "domestic" dog and "domestic" cat mean dogs and cats, in particular *Felis domesticus* and *Canis domesticus*.

The level of plasma vitamin E in a cat or dog can easily be determined. A representative example of determining plasma vitamin E level is described in the introductory portion of the examples.

The concentration of vitamin E in a product (solid or liquid or any other form) can easily be determined. This is also described in the introductory portion of the examples.

In the first aspect of the invention, the control diet may, instead, provide a total vitamin E to the animal of 15IU/400kcal.

Preferably, the administration of the vitamin E according to the first aspect of the invention is at a level of from 25IU/400kcal diet. Throughout this text, references to concentrations per kcal are to kcal total metabolisable energy intake. The determination of calorie density can be identified using Nutritional Requirements of Dogs (1985) National Research Council (U.S.) National Academy Press Washington DC, ISBN: 0-309-03496-5 or Nutritional Requirements of Cats (1986) National Research Council (U.S.) National Academy Press Washington DC, ISBN: 0-309-03682-8. Preferred levels for cats are from 30IU/400kcal, from 35IU/400kcal, from 40IU/400kcal, from 45IU/400 kcal, from 50IU/400 kcal, from 55IU/400kcal, up to about 100IU/400kcal or above. Preferred levels for dogs are from 30IU/400kcal, from 40IU/400kcal, from 45IU/400kcal, from 50IU/400kcal, from 55IU/400kcal, from 60IU/400kcal, from 65IU/400kcal, up to about from 100IU/400kcal or above.

For the first aspect of the invention, the method may include the administration of an amount of vitamin C (ascorbic acid).

Vitamin C is a water-soluble substance. It is synthesised *de novo* in both the domestic cat and the domestic dog. Because it is synthesised *in vivo*, the effect of vitamin C supplements in dog and cat has not previously been investigated. In particular, the effect of vitamin C supplementation in cat and dog, as a potential antioxidant and in combination with vitamin E supplementation has not been investigated.

The present invention shows that vitamin C levels in a cat or a dog can be increased by supplementation. This is demonstrated by an increase in plasma values following vitamin C supplementation. The increase in vitamin C levels can contribute to a reduction in free radicals and therefore a reduction in oxidative stress in the animal.

The vitamin C according to the first aspect of the invention may be in any form. It may be liquid, semi-solid or solid. Preferably it is a heat stable form such as a form of calcium phosphate.

The source of the vitamin C is not limiting. Preferred vitamin C sources include crystalline ascorbic acid (optionally pure), ethylcellulose coated ascorbic acid, calcium phosphate salts of ascorbic acid, ascorbic acid-2-monophosphate salt or ascorbyl-2-monophosphate with small traces of the disphosphate salt and traces of the triphophate salt, calcium phosphate, or for example, fresh liver.

The level of vitamin C in a product (solid, liquid or any other form) can easily be determined. This is described in the introductory part of the examples.

A further useful point in relation to the use of vitamin E in combination with vitamin C is their potential to act synergistically. This may be assisted by the fact that vitamin E is lipid soluble and vitamin C is water-soluble. Alpha-tocopherol is known to sit in the lipid membrane. Ascorbate and alpha-tocopherol, for example, interact at the interface between cell membranes or lipoproteins and water. Ascorbic acid rapidly reduces alpha-tocopherol radicals in membranes to regenerate alpha-tocopherol. The preferred concentration of vitamin C according to the first aspect of the invention is a level which preferably increases the plasma vitamin C level of an animal by up to about 25% (preferably 25% or more) in comparison with when the animal is fed a control diet, such that its total vitamin C consumption is (for both a cat or a dog) 5mg/400kcal diet. Levels of vitamin C which do not achieve this increase are still covered by the first aspect of the invention. Levels of vitamin C according to the first aspect of the invention include from 10, 12, 15, 17, 20, 22, 25, 27, 30, 32, 38, 40, 42, 48 up to about 50 mg/400kcal diet. Preferred levels for the cat are the above options from 10 to 48 mg/400kcal and for the dog, the above options from 12 to 50 mg/400kcal. Levels above 55 mg/400kcal provide no added benefit and are usually best avoided.

The first aspect of the invention may include the administration of an amount of taurine. The taurine may be in addition to, or instead of, the supplemented vitamin C described above.

Taurine is an unusual amino acid found in a wide variety of animal species. Taurine is an essential nutrient for the cat which, unlike the dog, is unable to synthesise taurine from precursor amino acids. It is thought that taurine protects cellular membranes from toxic components including oxidants. The increase in vitamin taurine levels in an animal diet can contribute to a reduction in free radicals and therefore a reduction in oxidative stress in the animal, in particular in combination with the other components of the invention.

The taurine according to the first aspect of the invention may be in any form. It may be powered, crystalline, semi-solid or liquid.

The source of the taurine is not limiting. Preferred taurine sources include aminoethylsulfonic acid (C2H7N03S). Sources may be natural or synthetic.

Suitable concentrations of taurine for use according to the first aspect of the invention are usually determined, to some extent as to the processing of the product (for example, whether the product is dry or canned). To maintain plasma taurine levels in the cat at the normal range (>60μmol/l), a canned (moist) diet must supply at least 39mg of taurine/kg body weight per day and a dry diet at least 19mg/kg body weight per day. The first aspect of an invention provides, for a product which is not subjected to a high temperature method (such as canning) a preferred level of from about 80mg/400kcal, more preferably from about 100, increasing even more preferably from 120, 150, 180, 200, 220, 250, 280, 300, 320, 350, 400 and above in mg/400kcal diet. In a product which is processed such as by high temperature, levels according to the invention are preferably from about 380mg/400kcal, more preferably from about 400,

increasing even more preferably from 420, 450,480, 500, 520, 550, 580, 600, 620, 650, 700 and above in mg/400kcal diet.

The concentration of taurine in a product (solid liquid or in any other form) can be easily determined. A representative method is described in the introductory portion of the examples. The *in vivo* feline status of taurine can be enhanced through dietary supplementation. The dose response effect of dietary taurine content can be measured by plasma levels. This is also described in the introductory portion of the examples.

The first aspect of the invention may further include the administration of an amount of a carotenoid. The carotenoid may be in addition to, or instead of, the supplemented vitamin C and/or the taurine as described above.

The carotenoids are a group of red, orange and yellow pigments predominantly found in plant foods, particularly fruit and vegetables, and in the tissues of animals which eat the plants. They are lipophilic compounds. Some carotenoids act as a precursors of vitamin A, some cannot. This property is unrelated to their antioxidant activity. Carotenoids can act as powerful antioxidants. Carotenoids are absorbed in varying degrees by different animal species. Carotenoids may be classified into two main groups; those based on carotenes and those based on xanthophylls (which include oxygenated compounds). Common carotenoids include; beta-carotene, alpha-carotene, lycopene, lutein, zeaxanthin and astaxanthin. Carotenoids are not proven to be essential nutrients in the feline or canine diet. Unlike humans and dogs, the cat is unable to convert the precursor beta-carotene into the active vitamin A form since the required enzyme necessary for this conversion is absent from the intestinal mucosa in cats (they do not possess the dioxygenase enzyme which is needed to cleave the carotene molecule).

This invention shows that carotenoids can be absorbed by the domestic cat and dog (to give an increased plasma concentration) and can contribute to a reduction in oxidative

stress. Further, the present invention has demonstrated that the carotenoids can be absorbed following their incorporation into a commercial product. As mentioned above, the components of the first aspect of the invention may act synergistically. Vitamin E is able to protect beta-carotene from oxidation and may have a sparing effect on beta-carotene. Vitamin E is thought to protect the chemical bonds of beta-carotene from being oxidised.

The source of the carotenoids is not limiting and can include natural and synthetic sources. In particular, the preferred source is a natural source and includes; marigold meal and lucerne meal (sources of lutein); tomato meal, red palm oil, tomato powder, tomato pomace/pulp (sources of beta-carotene and lycopene). Sources include oils high in carotenoid levels and pure manufactured carotenoids such as lutein, violaxanthin, cryptoxanthin, bixin, zeaxanthin, apo-EE (Apo-8-carotenic acid ethylester), canthaxanthin, citranaxanthin, achinenone, lycopene and capsanthin. Preferred levels of total carotenoids are from 0.01mg/400kcal, or from 0.2mg/400kcal or from 1mg/400kcal or from 2mg/400kcal.

The concentrations of the following carotenoids are preferably:
Beta-carotene: 0.01 to 1.5mg/400kcal, preferably 0.5 to 1mg/400kcal
Lycopene: 0.01 to 1.5mg/400kcal, preferably 0.5 to 1mg/400kcal
Lutein: 0.05 to 1.5mg/400kcal, preferably 0.5 to 1mg/400kcal.
In particular, the present invention provides for a combination of carotenoids in the first aspect of the invention.

Preferred sources of the combined carotenoids include;

Red Palm Oil and Marigold Meal

Tomato Powder, Marigold Meal and Lucerne

Tomato Pomace and Marigold Meal.

As described above, the invention includes vitamin E and optionally other components. Useful combinations of the components (preferably in a canned or dry petfood) include;

Vitamin E, vitamin C, taurine, red palm oil and marigold meal
Vitamin E, vitamin C, taurine, tomato powder, marigold meal and lucerne
Vitamin E, vitamin C, taurine, tomato powder and marigold meal
Vitamin E, vitamin C, taurine, tomato powder and lucerne
Vitamin E, taurine, tomato pomace and marigold meal.

A combination of the present invention is;

	Approx. active component mg/400kcal after production (Dry Product)	
Vitamin C	20mg ascorbic acid	
Vitamin E	50 IU	
Taurine	200mg	(500 mg in wet product)
Lutein	0.17mg	
Lycopene	0.03mg	
Beta-carotene	0.01mg	

A further useful combination of the present invention is:

Vitamin E	50IU/400kcal
Vitamin C	20mg/400kcal
Taurine	500mg/400kcal
Beta-carotene	0.5 to 1mg/400kcal
Lycopene	1mg/400kcal
Lutein	0.5 to 1mg/400kcal

Other useful components of the foodstuff according to the invention, include; trace minerals (not direct antioxidants, but function as cofactors within antioxidant metalloenzyme systems), selenium (an essential part of the antioxidant selenoenzyme, glutathione peroxidase), copper, zinc and manganese (forming an integral part of the antioxidant metalloenzymes Cu-Zn-superoxide dismutase and Mn-superoxide dismutase.

In accordance with the method of the first aspect of the invention, the components may be administered, or consumed, simultaneously, separately, or sequentially.

In accordance with a second aspect of the invention, there is provided a dog or cat foodstuff which delivers to said animal, a concentration of ingredients sufficient to increase the antioxidant status of the animal.

All preferred features of the first aspect of the invention also apply to the second. In particular all of the levels and preferred levels (including more preferred and most preferred levels) according to the first aspect also apply to the second.

Preferably, the dog or cat foodstuff provides an antioxidant status of greater than 20mg/l of vitamin E.

A third aspect of the invention provides a dog or cat foodstuff which provides a concentration of vitamin E at a level according to the first aspect of the invention. The concentration may be as stated according to the first aspect of the invention which provides the described percentage increases or the particular (including preferred) levels.

The dog or cat foodstuff according to the third aspect may also provide a concentration of vitamin C at a concentration also according to the vitamin C levels of the first aspect of the invention.

The dog or cat foodstuff of the third aspect may provide, in addition, or as an alternative to the vitamin C, a concentration of taurine at a concentration also according to the taurine levels of the first aspect of the invention.

The dog or cat foodstuff according to the third aspect may provide, in addition to the vitamin C and/or the taurine or as an alternative, a concentration of a carotenoid at a concentration also according to the carotenoid levels of the first aspect of the invention.

Preferred features of aspects one and two, also apply to the third aspect.

A fourth aspect of the invention provides a dog or cat foodstuff according to the third aspect of the invention, for use in the prevention or treatment of low antioxidant status in a dog or cat.

Preferred features of aspects one to three also apply to the fourth aspect.

A fifth aspect of the invention provides a dog or cat foodstuff according to the second, third, fourth or ninth aspects of the invention, for use in the prevention or treatment of any disorder which has a component of oxidative stress. The use is separately for the prevention or treatment of oxidative stress as a component of a "disease" or "disorder" (thus the disease or disorder may be reduced by alleviating (at least to an extent) a component of oxidative stress). Such disorders include; ageing, cancer, heart disease, atherosclerosis, arthritis, cataracts, inflammatory bowel disease, renal disease, renal failure, neurodegenerative disease and immunity (such as compromised immunity). Also included are prevention and treatment of oxidative stress caused by animal

vaccinations (often annually) and anaesthetics, which may also be used for annual procedures such as dental treatments (which may require general anaesthetic) and exposure to UV light or radiation. With respect to immune function, this is equally applicable to those subjects who have a compromised immune function due to age (e.g. growing animals or senescing animals) as well as those experiencing immunological challenge. The maintenance of a healthy immune response (as well as optimising or boosting an immune response) in animals who are clinically healthy is also included in this definition.

The immune system of vertebrate animals is much discussed in the art (for example "Immunology" by Roitt, Brostaff and Male, Gower Medical Publishing, London, New York, 1985). Immunological challenge includes infection, vaccination and other external factors such as anaesthesis (for example prior to surgery). It is an object of the present invention to provide a diet/foodstuff or supplement (and related aspects) which can be used to maintain, optimise or "boost" the immune system such that an improved immune response is given on an immunological challenge. An immune response can be monitored by measuring antibodies produced in response to a given antigen. Such knowledge and technology is standard in the art. An improved immune response may be represented by a higher level (titre) of circulating antibodies within a given time frame, a faster detected antibody response or maintenance of the circulating antibody titre for a longer period of time.

An improved immune response assists the animal during an immunological challenge and can be particularly useful for young animals, since young animals may not have a fully developed immune system. As young animals are often vaccinated, the present invention provides means by which an improved immune response can be given by the animal when vaccinated. The present invention is particularly useful for feeding to a dog prior to vaccination with vaccine antigens for distemper, parvovirus and/or adenovirus. The present invention is also useful for feeding to a dog for vaccine against rabies virus. The present invention is particularly useful for feeding to a cat

for vaccines or a combined vaccine against Feline Panleucopenia, Feline Calicivirus and/or Feline Herpesvirus. The present invention is also useful for feeding to a cat for a vaccine against Feline rabies virus. The length of time suitable for feeding prior to immune challenge depends on the animal in question and the immunological challenge. The foodstuff can be fed consistently. Periods of 8, 6, 4, 2 and 1 week prior to immune challenge are suitable. Longer periods are also suitable.

It is recognised that at periods in an animals life, such as in newborns, elderly animals or pregnant females or in periods of stress induced *via* malnutrition, infection or other environmental stresses, animals will be immunocompromised and as a result vaccines will be less efficacious. If an animal is clinically recognised as being immunosuppressed a decision may even be made not to vaccinate. Nutritional supplements according to the present invention can be shown to enhance the immune response even in healthy animals. The studies presented here show the benefits of nutritional supplements in enhancing humoral immune response to vaccinations in both normal healthy adult cats or dogs and other life stages of cats and dogs. If nutritional intervention can enhance what can already be regarded as an optimal immune response then it is logical to make changes to a diet that such supplementation will greatly improve the efficacy of vaccination in animals immunosuppressed for any variety of reasons.

The present invention also provides a method (suitable for a dog or a cat) for preventing or treating a component of oxidative stress or a disorder which has a component of oxidative stress, said method comprising feeding said dog or cat a foodstuff according to the present invention. The disorders are as described above. The invention also relates to a method for strengthening an immune response, in a dog or cat, to an immunological challenge, said method comprising feeding a foodstuff according to the present invention.

Preferred features of aspects one to four also apply to the fifth aspect.

A sixth aspect provides for the use of vitamin E, in the manufacture of a medicament/clinical or veterinary diet for the prevention or treatment of any cat or dog disorder which has a component of oxidative stress, or for the prevention or treatment of oxidative stress.

Preferred features of aspects one to five also apply to the sixth.

A seventh aspect of the invention provides for the use of vitamin E at a level of 25IU/400kcal or above, incorporated into a foodstuff as an *in vivo* antioxidant, in a dog or a cat. All preferred features of aspects one to six also apply to the seventh. In particular, the levels of vitamin E may be as the preferred levels for vitamin E set out for the first aspect of the invention.

An eighth aspect of the invention provides a method for making a foodstuff according to any of the second to fifth aspects of the invention the method comprising mixing together at least two ingredients of the foodstuff. One of the components will be the required level of vitamin E. The preferred form of the foodstuff is a petfood product and therefore the method for making the petfood product, in any form, comprises mixing together the ingredients for the petfood product and incorporating one or more of the components according to the invention. The components may be added at any time during the manufacture/processing of the foodstuff, including at the end, as the last step before packaging.

The product can be made according to any method known in the art, such as in Waltham Book of Dog and Cat Nutrition, Ed. ATB Edney, Chapter by A. Rainbird, entitled "A Balanced Diet", pages 57 to 74, Pergamon Press.

A ninth aspect of the invention provides a dog or cat foodstuff comprising vitamin C at a concentration of from 15mg/400kcal diet. The diet, foodstuff or supplement details are as those described for the previous aspects of the invention in relation to the vitamin C component to the extent that it comprises a vitamin C concentration of from 15 mg/400kcal diet. Features of aspects one to eight, as herein described may be individual or combined options together with the vitamin C concentration according to the ninth aspect of the invention. The ninth aspect of the invention provides a foodstuff useful for the prevention or treatment of a disorder which has a component of oxidative stress. Such disorders are also those as described above for the previous aspects of the invention. The inclusion of vitamin C in a dog or cat foodstuff is unique in as far as it relates to the concentrations of vitamin C stated and in particular or for the uses given.

The vitamin C concentrations range from 15mg/400kcal upwards. Preferred levels are those above 15mg/400kcal as set out above according to the preferred concentrations of vitamin C according to the first aspect of the invention. Because vitamin C is synthesised *in vivo* in both the domestic cat and the domestic dog it has never been of particular interest to consider introducing to a cat or dog supplemental levels of vitamin C via cat or dog food. However, the present invention shows that such a diet can be particularly useful, primarily for the production of a clinical diet/veterinary diet/medicament.

The present invention also provides for the use of vitamin C in a foodstuff for a dog or a cat. The use may be in the manufacture of a diet for the prevention or treatment of a disorder which has a component of oxidative stress or for the prevention or treatment of the oxidative stress component. Those disorders include cancer, ageing, heart disease, atherosclerosis arthritis, cataracts inflammatory bowl disease, renal disease, renal failure, neurodegenerative disease or compromised immunity, for example, an animal suffering from an infection. The present invention may also be used to treat or assist in the event of an immunological challenge in healthy animals. Such an

immunological challenge includes vaccination. Particular vaccinations are those described in the present text.

The present invention also relates to a method for the prevention or treatment, in a dog or cat, of a disorder which has a component of oxidative stress (or of the oxidative stress component) comprising feeding to said cat or dog a foodstuff according to the ninth aspect of the invention.

The present invention (as a twelfth aspect) also provides for the supplementation of a pet food with one or more of lycopene, vitamine E, vitamin C, beta carotene or taurine to treat or assist in the event of oxidative stress in an animal. The oxidative stress may be an immunological challenge. The oxidative stress may be present in a healthy animal or in an animal which is immunosuppressed. The animal is preferably as described for the first aspect of the invention. The immunological challenge may be vaccination, in particular vaccination against one or more of Feline Panleucopenia, Feline Calcivirus, Feline Herpesvirus, Feline Rabies, Canine Distemper, Canine Parvovirus, Canine Adenovirus or Canine Rabies.

All prefered features of the twelfth aspect of the invention, such as compositions/product types, etc., levels of lycopene, vitamine E, vitamin C, beta-carotene and/or taurine, sources or forms of these components, methods of treatment, assistance, prophylaxis and uses are as described in any one of the first to eleventh aspects as hereinbefore and hereinafter described.

The following figures are referred to in the examples section:

Figs. 1 and 2, which show levels of anti-parvovirus antibody titres with supplemented and unsupplemented diets, post vaccination.

Fig. 3, which shows an anti-distempter vaccine response with supplemented

and unsupplemented diets, post vaccination.

Fig. 4, which shows maintenance of anti-adenovirus antibody titres in dogs supplemented with an antioxidant cocktail.

Fig. 5 shows mean anti-calicivirus antibody in the units in cats supplemented with different antioxidant cocktails.

Fig. 6 shows concentration of NaCl at which 50% of cells exhibit haemolysis.

Fig. 7 shows mean anti-rabies antibody in the units in animals supplemented with antioxidants.

The invention will now be described with reference to the following non-limiting examples. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLES

Introductory Portion

This section describes, firstly, how blood samples may be taken for determination of vitamin E, vitamin C, taurine and carotenoids. Also described are methods for analysis of components in plasma and methods for measuring components in food. In addition to the details set out below, details regarding analytical procedures can be found in McDowell L.R. (1989) Vitamin E: In Vitamins in Animal Nutrition Chapter 4, page 96, Academic Press, UK.

Plasma and Whole Blood Taurine

Preparation of samples:

Blood samples are collected into heparinarised bottles from either the cephalic or jugular vein. Following mixing of the sample on a roller, the samples are kept on ice for transfer to the laboratory. Whole blood is then frozen at -20^0C until analysis. Alternatively for plasma measurement, plasma is extracted by centrifugation of blood samples (at 3500 rpm for 10 minutes at 0^0C). Plasma is frozen at -20^0C until analysis.

The analysis of Taurine in cat plasma/blood is carried out by taking the sample and precipitating out protein by reaction with sulpho-salicylic acid solution. The sample is then centrifuged and the supernatant liquor filtered.

Reference where plasma taurine has been measured in cats:
Earle, K.E. and Smith, P.M. (1991) The effect of dietary taurine content on the plasma taurine concentration of the cat. British Journal of Nutrition 66, 227-235.

Plasma Vitamin C

Preparation of samples:
Blood samples are collected into heparinarised light-protected (foil-wrapped) bottles from either the cephalic or jugular vein. Following mixing of the sample on a roller, the samples are kept on ice for transfer to the laboratory. Plasma is extracted by centrifugation of blood samples (at 3500 rpm for 10 minutes at O^0C). Plasma is frozen at -20^0C until next-day analysis. Samples are prepared under subdued lighting at all times.

1ml plasma extracted with 5ml extractant (15g metaphosphoric acid + 0.475g EDTA + 20ml glacial acetic acid in 500ml water) – the procedure is then the same as for product.

A preferred minimal dose of vitamin C to achieve an increase in plasma in cats is 20mg/400kcal. A preferred minimal dose of vitamin C tested to achieve an increase in plasma in dogs was 27mg/400kcal.

Plasma Vitamin E

Preparation of samples:

Blood samples are collected into heparinarised bottles from either the cephalic or jugular vein. Following mixing of the samples on a roller, the samples are kept on ice for transfer to the laboratory. Plasma is extracted by centrifugation of blood samples (at 3500 rpm for 10 minutes at 0^0C). Plasma is frozen at -20^0C until analysis.

Sample size = 250µl. The sample is extracted into hexane after the addition of tocopherol acetate as internal standard. The hexane is evaporated and the residue dissolved in methanol and injected onto the HPLC. Separation is achieved using a reverse-phase column with methanol as eluent with UV detection at 285nm.

A preferred minimal dose of vitamin E to achieve an increase in plasma in cats is 34 IU/400kcal. A preferred minimal dose of vitamin E tested to achieve an increase in plasma in dogs was 50IU/400kcal.

Carotenoid determination in plasma

Blood samples are collected into heparinarised light-protected (foil-wrapped) bottles from either the cephalic or jugular vein. Following mixing of the samples on a roller, the samples are kept on ice for transfer to the laboratory. Plasma is extracted by centrifugation of blood samples (at 3500 rpm for 10 minutes at 0^0C). Plasma is frozen at -80^0C until analysis. Samples are prepared under subdued lighting at all times.

The following two methods may be used to determine carotenoid concentration in plasma.

Method 1

The first method is to measure the major carotenoids of interest, with the exception of lutein and zeaxanthin which will not be separated using this method.

The method used to detect carotenoids is a variation of that of Craft, N.E. and Wise, S.A., Journal of Chromatography, 589, 171-176, (1992).

The extraction of carotenoids from plasma is achieved using a variation of that of Thurnham *et. al.* Clinical Chemistry, 34, 377-381, 1988.

Method 2

The second method is to separate lutein and zeaxanthin and to separate the different isoforms of the carotenoids.

The method used to detect the carotenoids is a variation of that of Yeum, Kyung-Jin., *et. al.* Am. J. Clin. Nutr, 64, 594-602, 1996.

The extraction of carotenoids from plasma is achieved using a variation of that of Thurnham *et. al.* Clinical Chemistry, 34, 377-381, 1988.

All extractions were carried out under subdued lighting, and all stock solutions of carotenoids were stored under argon.

Vitamin C – Food Product

Ascorbic acid is enzymatically oxidised to dehydro ascorbic acid which is condensed with o-phenylene diamine to the fluorescent quinoxaline derivative. The latter is separated from interfering compounds by reversed-phase HPLC with fluorimetric detection.

Vitamins A & E Food Product

The sample is hydrolysed with ethanolic potassium hydroxide solution and the vitamins extracted into petroleum ether. The petroleum ether is removed by evaporation and the residue is dissolved in propan-2-ol. The concentration of vitamin

A and E in the propan-2-ol extract is determined by reversed-phase liquid chromatography.

Free Taurine – Food Product

Free Taurine is that which is nutritionally available in a product.

The analysis of Free Taurine is carried out by taking the sample, adding dilute Hydrochloric acid. This is then macerated and transferred to a volumetric flask. A small amount is then taken and sulpho-salicylic acid is added to precipitate the protein. The sample is then centrifuged and the supernatant liquor filtered. The resulting solution is reacted with dansyl chloride and analysed by HPLC using fluorescence detection.

Carotenoids – Food Product

20-25g sample taken for analysis. Sample is saponified with 28% ethanolic potassium hydroxide for 30mins. At 90^0C under nitrogen and with pyrogallol as antioxidant. After cooling, the saponified extract is extracted with 2x250ml mixed ethers (pet. Ether/diethyl ether 1:1) and the organic phase is washed with water until neutral. The ether extract is evaporated at 35^0C under vacuum with BHT as antioxidant and the residue redissolved in the HPLC mobile phase. The carotenoids are determined using reverse phase HPLC using UV detection at 450nm.

In addition to the experimental work given, the invention was an indicator of improved health by decreasing the osmotic fragility of cat erythrocytes following feeding of the antioxidant cocktail to cats.

The ability of red blood cells (erythocytes) to withstand osmotic stress was tested. The method involved re-suspension of washed erythrocytes in solutions with different NaCl concentrations; these are incubated and then centrifuged. Haemoglobin is released from the cells according to their osmotic fragility. Results showed that

erythocytes of cats fed antioxidant cocktails of the invention showed a greater resistance against osmotic stress as significant lower concentrations of NaCl were required to induce the same level of heamolysis. The ability of erythocytes to tolerate situations of osmotic stress is an indicator of an improved physiological status.

Example 1

Influence of the antioxidant supplemented diet on the immunological status of cats

EXPERIMENTAL DESIGN:

48 normal healthy cats were fed a control diet (complete diet as per the reference section) for six weeks after which baseline measurements were taken. Cats were then allocated to either control or treatment age -matched groups and fed the supplemented diet described below. At week eight the animals were sampled in order to determine serum immunoglobulin concentrations. At week twelve immune parameters were measured and the cats were immunised (using a standard combined vaccine against Feline Panleucopenia, Feline Calicivirus and Feline Herpesvirus). At week eighteen final measurements were made post immunisation.

Supplemented diet: A wet diet supplemented with an antioxidant cocktail for a period of 30 weeks.

The cocktail was:

Vitamin E	501U/400kcal diet
Vitamin C	20mg/400kcal diet
Beta-carotene	0.5-1mg/400kcal diet
Lutein	0.5mg/400kcal diet
Taurine	200mg/400kcal diet
Lycopene	1mg/400mg kcal diet

METHODS USED:-

Assessment of peripheral blood mononuclear cellular (PBMC) proliferative response by mitogen induced lymphocyte transformation assay (MILT)

Peripheral blood mononuclear cells were isolated from heparinised blood by density gradient centrifugation on Histopaque 1077(Sigma). The cells were washed twice with phosphate buffered saline (PBS) and once with RPMI-1640 (Dutch modification) supplemented with 10 per cent heat inactivated fetal calf serum, 1 per cent penicillin/streptomycin and 2 per cent sodium pyruvate. Cell viability was assessed by the trypan blue exclusion test (Sigma).

Cells were cultured in triplicate at 1 x 10^5 per well in 96 well flat bottomed microtitre plates at 37^0C, with phytohaemagglutinin (PHA) (5 μg/ml)(Murex), concanavalin A (Con A) (7.5 μg/ml) and pokeweed mitogen (PWM)(1 μg/ml)(Sigma) for 96 hrs. Proliferation was measured by [^{3}H]-thymidine incorporation in counts per minute (CPM) (0.5 μCi/well) during the final 18 hrs of culture.

Analysis of lymphocyte subsets by flow cytometry

CD4 and CD8 positive cells are the most well characterised lymphocyte subsets in feline immunology and an adequate repertoire of these cells is indicative of a healthy immune system. The assay was performed using both purified lymphocytes and whole blood and a selection of various monoclonal antibodies (Mabs).

RESULTS

Assessment of PBMC proliferative response by mitogen induced lymphocyte transformation assay (MILT)

Table 8 shows the response of PBMC to the mitogens PHA, Con A and PWM prior to and post immune challenge Mitogen induced lymphocyte transformation assay (MILT) data showed no significant changes in proliferative response for either control or treatment groups. When an analysis of stimulation indices was undertaken there was a significant decrease in the treatment group in both the PHA stimulation index (S I) ($p<0.05$) and the Con A index ($p<0.001$) from pre to post-immunisation. There was no significant difference in the SI of the control group. The Pokeweed SI increased significantly from baseline to pre-immunisation in both groups ($p<0.05$) and decreased significantly in the treatment group post immunisation ($p<0.01$).

Table 1.

The response of PBMC to the mitogens PHA, Con A and PWM prior to and post immune challenge.

[^{3}H]thymidine incorporation (counts per min) CPM x 10^{-3} MEAN ± SEM
Stimulation index (S.I.) MEAN ± SEM

	Baseline		Pre-immunisation		Post-immunisation	
	standard [a] (n =22)	lara plus [b] (n = 23)	standard (n =22)	lara plus (n = 23)	standard (n =22)	lara plus (n = 23)
Unstimulated	20 ± 4	21.6 ± 4	27.2 ± 3.6	18.2 ± 3	30.9 ± 3.9	25 ± 2.6
PHA	38.8 ± 4	41.5 ± 6	33.7 ± 3.9	28.5 ± 3.2	38.8 ± 4	33.3 ± 3
S.I.	*2.01 ± 0.2*	*2.69 ± 0.5*	*1.38 ± 0.1*	*2.2 ± 0.25*	*1.8 ± 0.58*	*1.49 ± 0.14* *
Con A	29 ± 3	32.4 ± 4.5	31.4 ± 3.5	38 ± 5.9	37.9 ± 4	33 ± 3.6
S.I.	*1.55 ± 0.1*	*1.85 ± 0.2*	*1.38 ± 0.1*	*2.3 ± 0.1*	*1.81 ± 0.5*	*1.46 ± 0.15* ***
PWM	21.5 ± 2.5	22 ± 4	37 ± 4.2	29.7 ± 3	37.8 ± 4	29.7 ± 3.3
S.I.	*1.1 ± 0.1*	*1.25 ± 0.15*	*1.5 ± 0.2* *	*2.52 ± 0.4* *	*2.03 ± 0.7*	*1.26 ± 0.1* **

[a] control group, standard diet
[b] treatment group, test diet

* $P < 0.05$; ** $P < 0.01$;
*** $P < 0.001$

Analysis of lymphocyte subsets by flow cytometry

Table 2 shows T-cell relative subset counts and CD4+: CD8+ ratio pre and post immunisation. When CD4 and CD8 T-cell subsets were analysed there was a

significant increase in percentage of CD4 positive cells ($p<0.05$) in both groups and a significant increase in CD8 positive cells in both the control group ($p< 0.05$) and test group ($p<0.001$) post immunisation.

When the CD4 +: CD8 + ratio of lymphocytes was examined it was found to be decreased significantly in the control group ($p<0.001$) while remaining constant in the treatment group post immune challenge. When examining age relationships there was a trend towards a decreasing CD4+: CD8+ ratio with increasing age in the control group prior to immunisation ($r = -0.483$, $p<0.05$).

Table 2

T-cell relative subset counts and CD4+: CD8+ ratio pre and post immunisation, MEAN ± SEM

	Pre immunisation		Post immunisation	
	standard [a] (n = 21)	lara plus [b] (n = 23)	standard (n = 21)	lara plus (n = 23)
CD4 positive percentage	22.6 ± 1.1	20.9 ± 0.7	25.1 ± 1.6 *	24.5 ± 1.3 *
CD8 positive percentage	17.2 ± 0.1	15.4 ± 1.3	22.3 ± 1.2 *	19.6 ± 1.6 **
CD4:CD8 ratio	1.42 ± 0.1	1.57 ± 0.1	1.17± 0.1**	1.43 ± 0.1

[a] control group, standard diet

[b] treatment group, test diet

* P < 0.05 ; ** P < 0.001

The observed difference in SI of PWM stimulated cells from cats fed the supplemented diet (Table 1) suggests that there is a beneficial upregulation of CD2, an activation marker of T-cells.

The results on Table 1 show clearly the beneficial effects of the supplemented diet on the CD4:CD8 ratios of cats post-vaccination. The CD4:CD8 ratio in the supplemented cats was maintained post vaccination compared to the control group. This maintenance is mainly due to an increase in CD4.

These facts show beneficial effects of the supplement upon the immune response of cats.

Example 2

Effects of an Antioxidant Cocktail on Specific Antibody Responses of Young Dogs

- Litters of Labrador and Greyhound Puppies were separated into two age and sex – matched groups.
- One group of each breed had their standard diet (complete, as per the reference section) supplemented with a cocktail (details given below), the other two groups (one of each breed), remained on an unsupplemented diet.

Antioxidant Cocktail-

alpha-tocopherol	50mg / 400kcal
ascorbate	20mg /400kcal dry (40mg if wet)
beta-carotene	0.5mg /400kcal
lutein	0.5mg /400kcal
taurine	200mg /400kcal dry (500mg if wet)

- Supplement was administered for up to a maximum of four weeks prior to vaccination.

- All of the puppies were vaccinated according to routine vaccination procedures (vaccines included Parvo-virus and Distemper).
- Antibody levels to vaccine antigens were measured for all puppies.
- Some of these results are shown on Figures 1, 2 and 3.
- These results clearly indicate that puppies receiving a supplement of the antioxidant cocktail will mount a faster response to specific antigens such as are introduced *via* a vaccine or which may be introduced through exposure to an infectious agent.
- These results show that the antioxidant cocktail has a highly beneficial effect on the immune response of young animals.

Example 3

Beneficial Effects of Antioxidant Cocktail on the Maintenance of a Vaccine Response in Adult and Senior Dogs

- Two groups of dogs were age, sex and breed matched.
- Both groups were further matched in accordance to when they had previously been vaccinated (prior to the start of the study).
- One group was fed a diet supplemented with an antioxidant cocktail (details given below), the other group remained an unsupplemented control.

Table 3

Antioxidant Content of Diet	Supplemented Test Diet	Control Diet
Vitamin E	52.41 IU/400 kcal	4.81 IU/400 kcal
Vitamin C	65.9 mg/400 kcal	2.48 mg/400 kcal
Taurine	0.16%	0.054%

Carotenoids		
-Cis Beta-carotene	11.07 ug/400 kcal	<10.96 ug/400 kcal
-Trans Beta-carotene	33.21 ug/400 kcal	21.91 ug/400 kcal
-Trans Alpha-carotene	<11.07 ug/400 kcal	10.96 ug/400 kcal
-Cis Alpha-carotene	<11.07 ug/400 kcal	<10.96 ug/400 kcal
-Lutein	0.996 mg/400 kcal	0.877 mg/400 kcal
-Lycopene	<11.07 mg/400 kcal	<10.96 ug/400 kcal
-Xeaxanthian	1.22 mg/400 kcal	1.32 mg/400 kcal

- After a period of six months on the supplemented diet the dogs had their circulating anti-adenovirus antibody titre measured. Results are shown on the graph in Figure 4.

- These results show that animals fed a diet containing an antioxidant cocktail are better able to maintain vaccine induced antibodies over time than are unsupplemented dogs.

Example 4

Effects of invention compositions on anti-calicivirus vaccine response in senior cats

Four groups of senior cats (n =3), were maintained on antioxidant supplemented diets for a period of 1 year. Details of the diets are given below:-

Diet 1 Containing lycopene and enhanced levels of vitamin E, beta-carotene, taurine and lutein compared with control and competitor diets

Diet 2 Containing red palm oil and enhanced levels of vitamins E and C, taurine, beta-carotene and lutein

Diet 3 Containing no enhanced levels of antioxidants or red palm oil

Diet 4 Competitor Diet

The base diet is represented by a complete balanced diet as described in the reference section.

Table 4 Dietary supplement contents of each of the four diets

DIET	Vitamin C [mg]	Vitamin E [IU]	Taurine [g]	Lutein [µg]	Red Palm Oil (g)	Trans-β-carotene (µg)	Lycopene (µg)
1	0.99	74.8	0.167	1142	-	57.12	19.04
2	37.08	98.55	0.176	7953	3.34	191.64	<9.6
3	0.97	56.97	0.118	881	-	47.38	<9.6
4	1.02	12.62	0.106	67	-	<9.6	<9.6

Amounts are given as /400kcal

A serum sample was taken from each of the cats prior to their annual booster vaccination, and then at 7 and 14 days after vaccination. Antibody titres to calicivirus were measured.

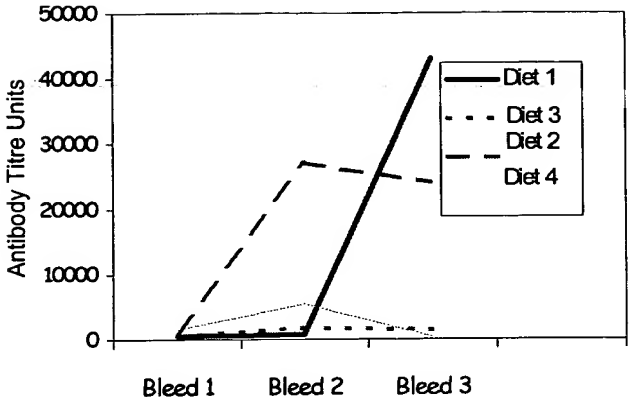


Figure 5 - Mean anti-calicivirus antibody titre units

The groups of cats which were fed on either Diet 1 or Diet 2 showed a marked increase in antibody response 7 and 14 days after vaccination respectively. In comparison the cats which had been fed on either Diets 3 or 4 did not shown an increase in antibody response even after 14 days post vaccination.

These results indicate the benefits of antioxidant supplements on increasing the efficacy of an anti-calicivirus vaccine response in animals, particularly senior animals.

Example 5

Effects of invention compositions on the membrane fragility of cat erythrocytes

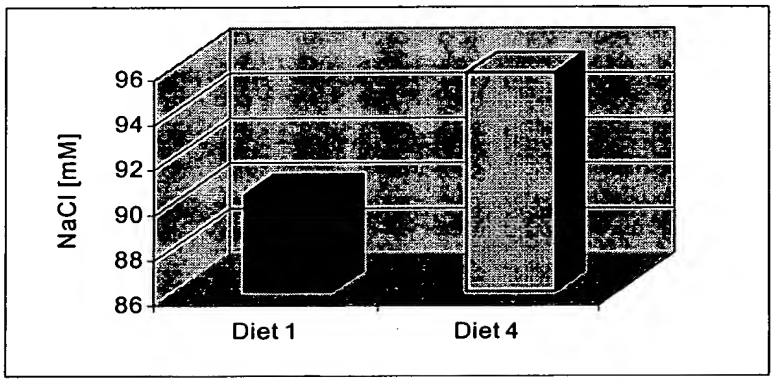
While animals can maintain either a high number of active, antibody producing B cells and/or high numbers of circulating memory T cells, they will remain better protected against specific antigen assault. However, even in a healthy immune system the number of effective, primed immune cells is in constant decline due to both necrosis and apoptosis. Following on from this, where immune cells are better able to withstand assault, avoiding destruction, an effective immune response is maintained

for longer. The study presented here shows that nutritional intervention has the ability to positively influence the membrane fragility of cells rendering them less susceptible to lysis.

Erythrocytes (used as an acceptable indicator of the situation in other circulating blood cells), were taken from each of the four groups previously described (Table 5). The ability of erythrocytes to withstand osmotic haemolysis was measured.

Osmotic haemolysis was induced by incubating the cells in decreasing concentrations of sodium chloride (NaCl). The theory behind this assay being that the lower the NaCl concentration the cells can withstand, the stronger their membrane stability. As well as having important implications in survival time, the integrity of a cellular membrane is crucial in cellular signalling, an important factor in effective immune function.

Figure 6 – Concentration of NaCl at which 50% of cells exhibit haemolysis



The results from this test (Figure 6), indicate that the erythrocytes taken from cats fed Diet 1 have greater membrane stability than erythrocytes from cats fed Diet 4. This implicates a role for nutritional intervention in the maintenance of cellular integrity and thus maintenance of an effective immune response.

Example 6

Effects of individual components of invention compositions on anti-rabies vaccine response in cats

Having previously shown that antioxidant cocktails are efficacious in enhancing humoral aspects of specific vaccine responses, individual ingredients of these cocktails were then examined for their effects on the humoral response using a rabies vaccination.

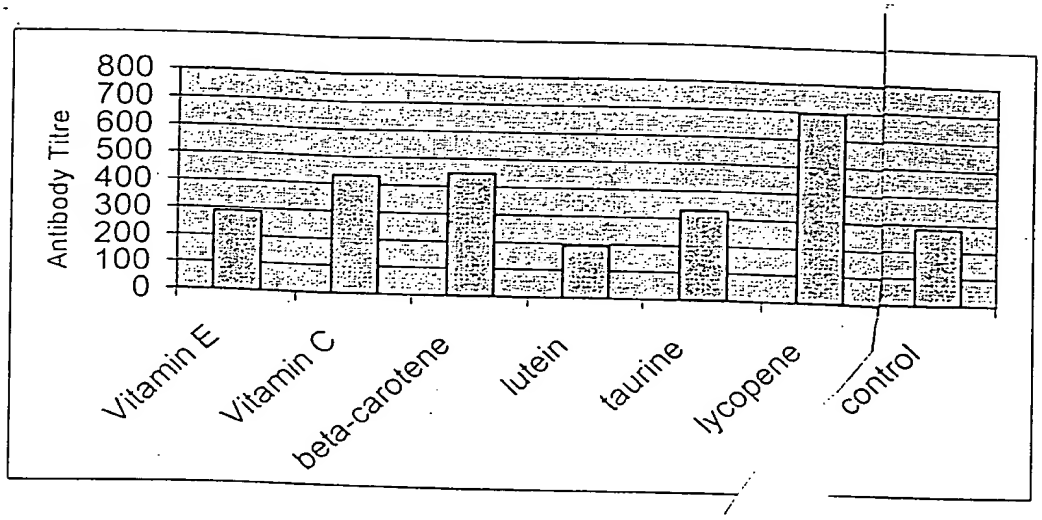
Eight groups of healthy adult cats (n=5) were orally supplemented once daily with dietary antioxidants at the levels described below (Table 5). The base diet was a complete diet as described in the reference section.

Table 5 – Details of dietary supplements as given to each group of cats

Group	Dietary Supplement	Amount (mg/400kcal)
1	Vitamin E	100
2	Vitamin C	80
3	beta-carotene	20
4	Lutein	20
5	Taurine	500
6	Lycopene	20
Control	Standard (Unsupplemented Diet)	n/a

These cats were vaccinated with a standard anti-rabies vaccine and specific antibody titres were measured. These results are shown on Figure 7.

Figure 7 – Mean anti-rabies antibody titre units



The results shown above indicate animals being fed a number of antioxidant supplements have a stronger response to immune challenge than unsupplemented controls. This effect is particularly marked in animals being fed enhanced levels of lycopene, although the reponse is also greater in animals being supplemented with vitamins E and C, beta-carotene and taurine.

Reference Diet Section

Nutritionally complete diet

A complete diet for foodstuff, especially a nutritionally complete petfood (or diet) is a diet which meets all the nutritional requirements of the individual animal's lifestyle and lifestage.

The diet or foodstuff can be made according to any method known in the art, such as

in Waltham Book of Dog and Cat Nutrition, Ed. ATB Edney, Chapter by A. Rainsbird, entitled "A Balanced Diet" in pages 57 to 74, Pergoren Press Oxford.

The following shows a composition of a complete balanced diet according to the Examples.

Ingredient	Inclusion
Rice	24.9%
Whole corn	18.8%
Whole grain wheat	12.2%
Chicken by-product meat	18.7%
Corn gluten meal	9.5%
Brewers yeast	1.7%
Dried egg	0.8%
Non-iodinised salt	0.7%
Vitamin premix	3.4%
Sunflower oil	0.5%
Beef tallow	4.9%
Poultry viscera	4.4%

Analytical profile – moisture 8.2%, protein 26.4%, fat 10.4%, ash 7.1%, fibre 2.2% (the remainder being made up of nitrogen-free extract (mainly carbohydrate)).

Claims:

1. A method for increasing the plasma vitamin E level in a cat or dog, the method comprising the step of administering to said cat or dog, an amount of Vitamin E sufficient to increase the plasma vitamin E level.

2. A method, as claimed in claim 1, further comprising administering to said cat or dog, an amount of vitamin C.

3. A method, as claimed in claim 1 or claim 2, further comprising administering to said cat or dog, an amount of taurine.

4. A method, as claimed in claim 1 or claim 2, further comprising administering to said cat or said dog, an amount of vitamin C and an amount of taurine.

5. A method as claimed in any one of claims 1 to 5, further comprising administering to said cat or dog, an amount of a carotenoid.

6. A method as claimed in any one of claims 2 to 6, wherein the components are administered simultaneously, separately, or sequentially.

7. A dog or cat foodstuff which delivers to said animal, a concentration of ingredients sufficient to increase the antioxidant status of the animal.

8. A dog or cat foodstuff which provides a concentration of vitamin E at a level of 25IU/400kcal diet or above.

9. A dog or cat foodstuff as claimed in claim 10, which provides a concentration of vitamin C at a level of 10mg/400kcal or above.

10. A dog or cat foodstuff as claimed in claim 10, which provides a concentration of taurine at a level of 80mg/400kcal or above.

11. A dog or cat foodstuff as claimed in claim 10, which provides a concentration of vitamin C at a level of 10mg/400kcal, or above and which provides a concentration of taurine at a level of 80mg/400kcal or above.

12. A dog or cat foodstuff as claimed on any one of claims 7 to 11, which provides a concentration of a carotenoid.

13. A dog or cat foodstuff as claimed in any one of claims 7 to 12, for use in the prevention or treatment of low antioxidant status in a dog or cat.

14. A dog or cat foodstuff as claimed in any one of claims 7 to 12, for use in the prevention or treatment of any disorder which has a component of stress.

15. A dog or cat foodstuff as claimed in claim 14, wherein the disorder is any one or more of; cancer, ageing, heart disease, atherosclerosis, arthritis, cataracts, inflammatory bowel disease, renal disease, renal failure, neurodegenerative disease or compromised immunity.

16. A dog or cat foodstuff, as claimed in any one of claims 7 to 14 for use in treating or assisting a cat or a dog in response to an immune challenge.

17. A foodstuff as claimed in claim 16, wherein the immune challenge is vaccination, in particular against Feline Panleucopenia, Feline Calicivirus, Feline Herpesvirus, Feline Rabies, Canine Distemper, Canine Parvovirus, Canine Adenovirus and/or Canine Rabies.

18. A method for preventing or treating a dog or cat suffering from a disorder which has a component of oxidative stress comprising feeding to said dog or cat a foodstuff as claimed in any one of claims 7 to 12.

19. A method of maintaining optimising or boosting an immune response to an immunological challenge in an animal comprising feeding said animal a foodstuff as claimed in any one of claims 7 to 12.

20. Use of vitamin E, in the manufacture of a medicament for the prevention or treatment of low antioxidant status in a dog or cat.

21. Use of vitamin E, in the manufacture of a clinical diet for the prevention or treatment of any disorder which has a component of oxidative stress.

22. Use of vitamin E, as claimed in claim 21, for maintaining, optimising or boosting an immune response, in a cat or a dog, in response to an immunological challenge.

23. Use of vitamin E, incorporated into a foodstuff as an *in vivo* antioxidant, in a dog or cat.

24. A method for making a foodstuff as claimed in any one of claims 7 to 14 or 21, the method comprising mixing together at least two ingredients of the foodstuff.

25. A dog or cat foodstuff comprising vitamin C at a concentration of 15mg/400kcal or above.

26. Use of vitamin C in the manufacture of a dog or cat foodstuff for the prevention or treatment of a disorder which has a component of oxidative stress.

27. Use of vitamin C as claimed in claim 26, wherein the disorder is any one or more of, cancer, ageing, heart disease, atherosclerosis, arthritis, cataracts, inflammatory bowel disease, renal disease, renal failure, neurodegenerative disease or compromised immunity.

28. Use of vitamin C as claimed in claim 26 for treating or assisting in response to an immunological challenge, such as vaccination, in particular vaccination against Feline Panleucopenia. Feline Calicivirus, Feline Herpesvirus, Feline Rabies, Canine Distemper, Canine Parvovirus, Canine Adenovirus and/or Canine Rabies.

29. A method for prevention or treatment in a cat or a dog of a disorder which has a component of oxidative stress comprising feeding said cat or dog a foodstuff as claimed in claim 25.

30. A dog or cat foodstuff as claimed in claim 7 which comprises:

alpha-tocopherol
beta-carotene
lutein and
taurine.

31. A dog or cat foodstuff as claimed in claim 30 which also comprises:
lycopene and/or ascorbate and/or red palm oil.

32. A dog or cat foodstuff as claimed in claim 30, wherein the components present as follows:

alpha-tocopherol:	from 25IU/400kcal
ascorbate:	from 5mg/kcal
beta-carotene:	from 0.01mg/400kcal

lutein:	from 0.05mg/400kcal
taurine:	from 80mg/400kcal

33. A dog or a cat foodstuff as claimed in claim 32 further comprising lycopene at a concentration of from 0.01mg/400kcal.

34. The use of one or more of lycopene, vitamin E, vitamin C, beta-carotene and taurine in the manufacture of a pet food product for treating or assisting in response to oxidative stress in a domestic dog or cat.

35. A method for treating or assisting a domestic dog or cat in response to oxidative stress comprising feeding to said animal (preferably in need thereof) a pet food product supplemented with one or more of lycopene, vitamine E, vitamine C, beta-carotene or taurine.

36. The use or a method, as claimed in claim 34 or claim 35 respectively, wherein the oxidative stress is an immunological challenge.

37. The use or a method as claimed in claim 36, wherein the immunological challenge is vaccination.

38. The use or a method as claimed in any one of claims 35 to 37, wherein the animal is healthy.

39. The use or a method as claimed in any one of claims 35 to 37, wherein the animal is immunosuppressed.

40. The use or method as claimed in claim 36, wherein the vaccination is against one or more of Feline Panleucopenia, Feline Calicivirus, Feline Herpesvirus,

Feline Rabies Virus, Canine Distemper, Canine Parvovirus, Canine Adenovirus, or Canine Rabies Virus.

FIG.1

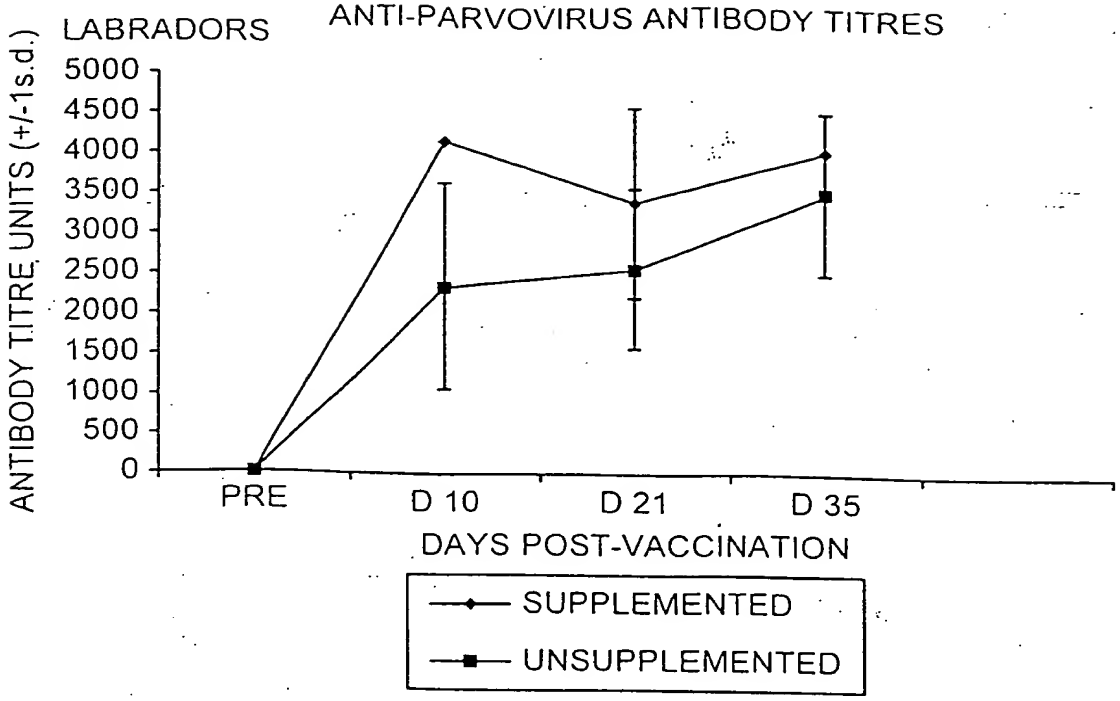
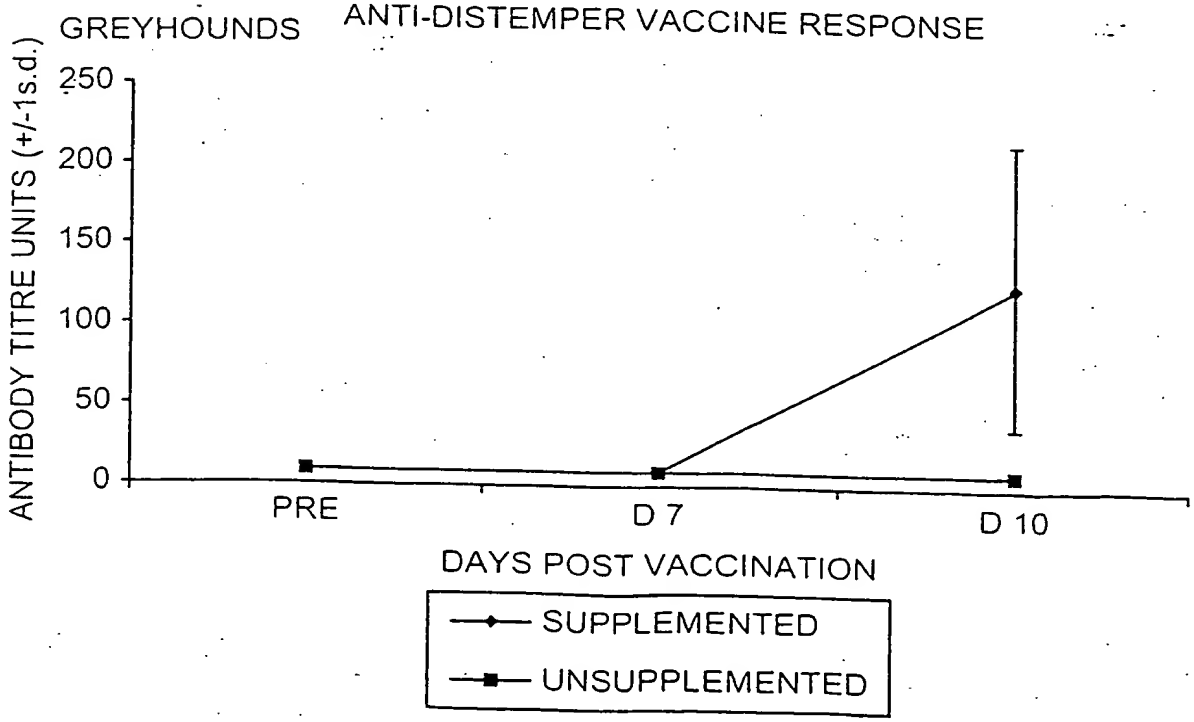

LABRADORS
ANTI-PARVOVIRUS ANTIBODY TITRES
ANTIBODY TITRE UNITS (+/-1s.d.)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
PRE
D 10
D 21
D 35
DAYS POST-VACCINATION
SUPPLEMENTED
UNSUPPLEMENTED

FIG. 2


GREYHOUNDS
ANTI-DISTEMPER VACCINE RESPONSE
ANTIBODY TITRE UNITS (+/-1s.d.)
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DAYS POST VACCINATION
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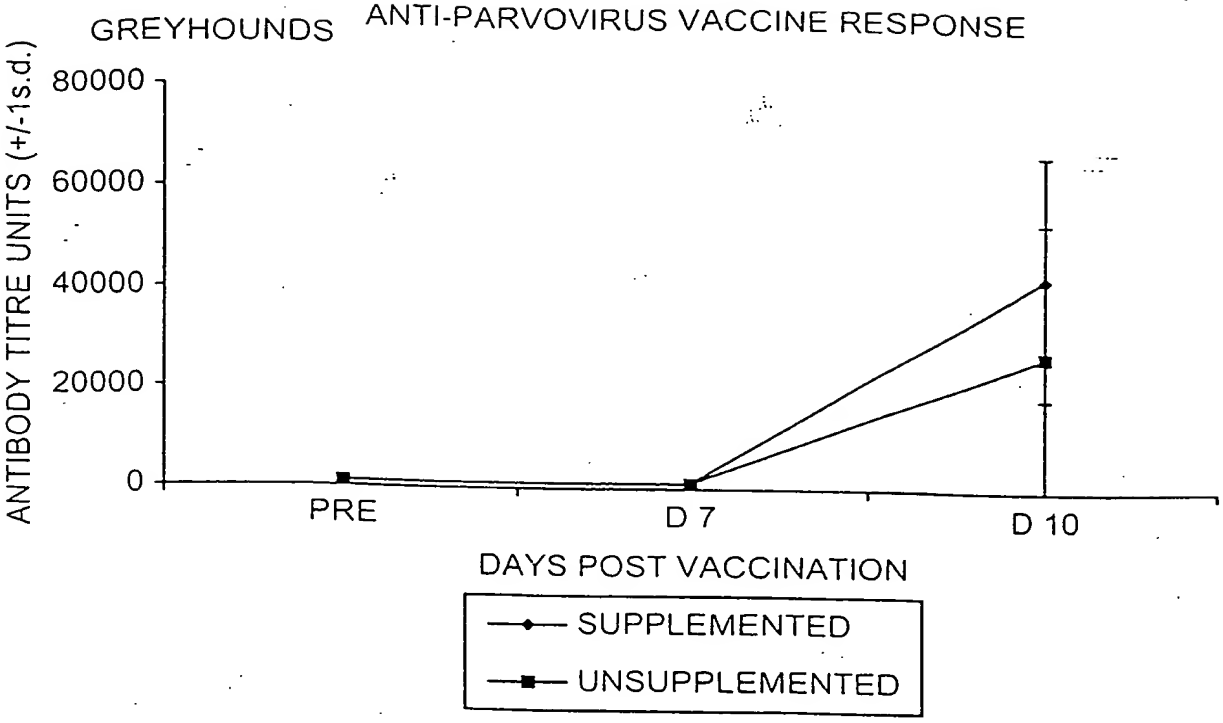
FIG. 3


GREYHOUNDS
ANTI-PARVOVIRUS VACCINE RESPONSE
ANTIBODY TITRE UNITS (+/-1s.d.)
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60000
40000
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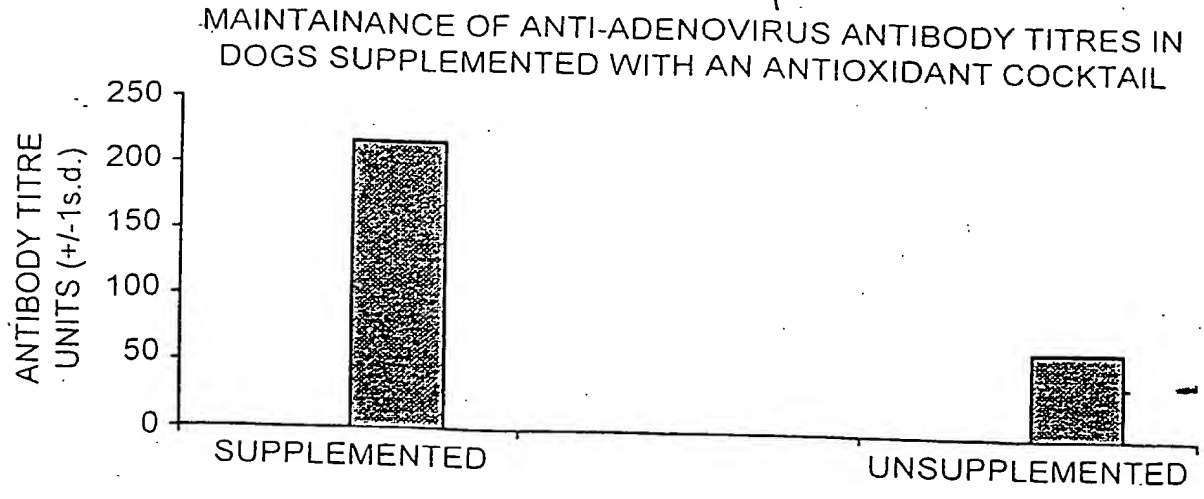
PRE
D 7
D 10
DAYS POST VACCINATION
SUPPLEMENTED
UNSUPPLEMENTED

FIG. 4


MAINTAINANCE OF ANTI-ADENOVIRUS ANTIBODY TITRES IN DOGS SUPPLEMENTED WITH AN ANTIOXIDANT COCKTAIL
ANTIBODY TITRE UNITS (+/-1s.d.)
250
200
150
100
50
0
SUPPLEMENTED
UNSUPPLEMENTED